SOIL NITROGEN POOLS, FORMS, AND LOSSES IN MATURE MISCANTHUS X GIGANTEUS UNDER NITROGEN FERTILIZATION TREATMENTS

BY

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THESIS

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Abstract

Miscanthus x *giganteus* is temperate climate dedicated energy crop with high biomass yield potential cultivated in the United States and Europe. Previous studies in young stands of *Miscanthus* x *giganteus* have shown little effect of nitrogen (N) fertilizer on yield production but have shown increased nitrate leaching and nitrous oxide (N_2O) emissions. The effect of this crop on soil organic matter, mineralization of N, and available carbon has not been fully determined, especially after many years of production. This study monitored 10x10 m plots of *Miscanthus* x *giganteus* replicated four times across a range of soil types (Mollisols, Ultisols and Alfisols) in Illinois, Kentucky, Nebraska, and New Jersey that were established in 2008. Some of the sites were replanted in 2009 after a severe winter and Virginia was planted in 2010. Urea fertilizer was added annually (0, 60, and 120 kg N ha^{-1} yr^{-1}) each spring. Above-ground biomass yields, nitrate and ammonium leaching, soil labile carbon (POX-C), soil potential mineralization of N (AnaN), and soil microbial activity (FDA) were evaluated during 2013 and 2014, building on previous work on these sites during the establishment phase.

Biomass yields were generally large at all sites (15 to 20 Mg ha^{-1} yr^{-1}) with greatest yields at Illinois and Nebraska (the two sites with Mollisols). Nitrogen fertilizer improved yields, however, it also greatly increased nitrate losses through leaching at all sites each year of the study. These losses were still increasing in the 6^{th} and 7^{th} years at 3 of the 5 sites and were higher with the 120 kg N ha^{-1} yr^{-1} treatment compared to the 0 or 60 kg N ha^{-1} yr^{-1}.

Potential soil mineralization of N and labile carbon were not affected by fertilization treatments in this study but sites with greater potential mineralization and labile carbon showed a greater

microbial activity according to FDA results. However, it would be necessary to compare results of this analysis periodically to document changes in microbial community composition. Finally, there were few significant changes in soil total C and N at either depth, suggesting no major changes in soil organic matter after 6 years of production.

Mature stands of *Miscanthus* x *giganteus* showed improvements in biomass production with the application of N fertilizer in this study but did not have an effect on POX-C or potential mineralization of N. In addition, a negative environmental impact of fertilization was shown as N losses through leaching which increased with fertilization rate.

Table of Contents

List of Tables v

List of Figures vi

Chapter 1: Introduction 1

Chapter 2: Results and Discussion 20

Chapter 3: Conclusions 52

References 55

List of Tables

Table 1.Results (P values) of the effects of the fixed variables of the PROC MIXED model.. 24

Table 2. Means and standard error of the effect of fertilization treatment and site on biomass production (Mg ha-1).. 25

Table 3. Means and standard error of the effect of fertilization treatment and site on nitrate and ammonium leaching kg N ha-1 yr-1. 32

Table 4. Means and standard error of the effect of fertilization treatment and site on Total C and N (%). 38

Table 5. Total C and N at 0-10 and 10-30 cm soil depths from all sites. Letters indicate significance different of means within a site and depth (α=0.05).. 39

Table 6. Means and standard error of the effect of fertilization treatment and site on AnaN, POX-C and FDA. 43

Table 7. Total monthly precipitation (mm) at each site from 2008 to 2015................................ 49

List of Figures

Figure 1. Map of Illinois plots with fertilization treatments including fertilization treatments in units of kg N ha-1 yr -1. 10

Figure 2. Map of Kentucky plots with fertilization treatments including fertilization treatments in units of kg N ha-1 yr -1. 11

Figure 3. Map of Nebraska plots with fertilization treatments including fertilization treatments in units of kg N ha-1 yr -1. 12

Figure 4. Map of New Jersey plots with fertilization treatments including fertilization treatments in units of kg N ha-1 yr -1. 13

Figure 5. Map of Virginia plots with fertilization treatments including fertilization treatments in units of kg N ha-1 yr -1. 14

Figure 6. Harvested biomass yields (Mg ha-1) from 2009 to 2015 in Kentucky and New Jersey. Standard errors are shown. Letters represent a significant difference between fertilization treatments. 26

Figure 7. Harvested biomass yields (Mg ha-1) from 2009 to 2015 in Kentucky and New Jersey. Standard errors are shown. Letters represent a significant difference between fertilization treatment. 27

Figure 8. Nitrate and ammonium leaching in Illinois by fertilizer rate in Illinois from 2009 to 2015 with standard errors. Letters indicate significant difference between treatments. 33

Figure 9. Nitrate and ammonium leaching in Kentucky by fertilizer rate from 2012 to 2014 with standard errors. Letters indicate significant difference between treatments. 34

Figure 10. Nitrate and ammonium leaching in Nebraska, New Jersey, and Virginia by fertilizer rate from 2012 to 2015 with standard errors. Letters indicate significant difference between treatments. 35

Figure 11. POX-C at 0-10 and 10-30 cm soil depths. Error bars represent the standard error. Letters indicate significance different of means between years... 40

Figure 12. Relationship between potential N mineralization and fluorescein concentration. 44

Figure 13. Average potential mineralizable N at soil 0-10 and 10-30 cm soil depths by sampling year. Error bars represent the standard error and letters significant difference between years. 45

Figure 14. Soil temperature at 10 cm in all sites from 2012 to 2015. 48

Figure 15. Volumetric soil water content at 10, 30, and 50 cm depths and daily precipitation at Nebraska and New Jersey from 2013 to 2015. 50

Figure 16. Volumetric soil water content at 10, 30, and 50 cm depths and daily precipitation at Illinois, Kentucky, and Virginia from 2013 to 2015... 51

Chapter 1: Introduction

The production of dedicated perennial biofuel crops such as *Miscanthus* x *giganteus* with minimal agricultural inputs and large biomass yields is a challenge to agronomists. The current energy demands from a fast growing global population has put pressure on the transportation biofuel industry to provide a solution that is environmentally friendly, yet does not interfere with food production. *Miscanthus* x *giganteus* could be a part of that solution, but there are many unknowns related to long-term production. Changes in and loss of soil carbon (C) and nitrogen (N) during the production of *M.* x *giganteus* have to be considered for the production of the crop and field measurements are essential for observing and reducing potential negative impacts to the environment.

In 2007, the United States government passed the Renewable Fuels Standard (RFS) as part of the Energy Independence and Security Act (EISA) and established the goal of producing 36 billion gallons of biofuels by 2022. With the use of corn grain ethanol, cellulosic biofuels, biodiesel and other advanced biofuels, the United States would be able to replace 30% of gasoline consumption. The production of bioenergy sources is subject to management practices and decisions that can affect the greenhouse gas emission budgets, potentially reducing soil C and N pools, and increasing losses of C and N through leaching and gas emissions (Davis et al., 2015).

Miscanthus x *giganteus* has been identified as a viable option for the production of biomass in some countries (Heaton et al., 2010), but the response of *M.* x *giganteus* stands to N fertilization rates is a critical factor in determining the environmental sustainability of this species as a biofuel crop (Arundale et al., 2014a). Even though it is a perennial grass requiring less N than annual crops and having the capacity to retranslocate N reducing the amount of N harvested in biomass, the potential increase in yields with N fertilization in developing and mature stands could have negative environmental impacts.

The perennial characteristics of *M.* x *giganteus* allow continuous production (no tillage needed after establishment with annual biomass harvests) for more than 15 years without replanting (Heaton et al., 2010). This clearly can provide environmental advantages over conventional annual agriculture production of a crop such as corn, which requires large inputs and is typically tilled annually. Because *M.* x *giganteus* can retranslocate N to the roots during senescence, before harvest in late fall or winter, this minimizes the amount of N harvested (Davis et al., 2013b; Smith et al., 2013). Production of *M.* x *giganteus* also seems to have rapid effects on soil mineralizable N pools, with increases documented in two studies (Davis et al., 2013a, 2015). This change in soil N availability could have a major role in long-term production and yields of the plant which is important in determining the potential of this biomass crop as part of the solution for sustainable production of fuels around the world.

Miscanthus species are rhizomatous grasses with a C4 photosynthetic pathway that were originally from Asia (Heaton et al., 2010). Some of the advantages of producing this type of grass are that it requires minimal fertilizer inputs compared to annual crops reducing the costs of fertilizers and the carbon foot print of its production (Heaton et al., 2010; Dohleman et al., 2012). One of its species, the hybrid *M.* x *giganteus*, is known for its capacity for high yields, sometimes producing over 20 t dry matter ha^{-1} yr^{-1} and its great adaptability capacity (Lewandowski et al., 2000; Heaton et al., 2010). Bohemel et al. (2008) considered this lignocellulosic biofuel as the most efficient crop energy wise (Bohemel et al., 2008) and Clifton-Brown et al. (2007) highlighted its extremely high potential for C storage due to the large quantities of rootstock.

Wang et al. (2012) suggested that *M.* x *giganteus* biomass feedstock yields could be improved by 40% in Illinois with N fertilization due to increases in canopy leaf area. However, the yield increase produced with fertilization is not as great as with corn or other crops (Arundale et al., 2014a). In fact, considering the economic return from fertilization and the relatively high yields of *M.* x *giganteus* without fertilizer, the amount of N fertilizer needed has been found to vary widely at different sites and conditions. In Urbana, Illinois trials on similar soils and with the same N fertilization rate, *M.* x *giganteus* had no response to the addition of the N fertilizer, whereas corn yields were doubled (Arundale et al., 2014a).

Long-term studies of *M.* x *giganteus* in Europe have shown that typically during the first three years after establishment yields increase each year (Lewandowski et al., 2000), which is the time commonly considered necessary to achieved ceiling annual yields (Arundale et al., 2014b). A Lesur et al. (2013) model suggested that the highest yield in a *M.* x *giganteus* plantation is obtained at 8.5 years after planting, later than Arundale et al. (2014a) estimations, but this could depend on the management practices and characteristics of the site. Some of these determining factors could be attributed to the planting method, planting density or even latitude and weather conditions.

There is even less understanding of long-term yields of *M.* x *giganteus*. Lesur et al. *(*2013) observed a declining trend in yields after several years, but this hypothesis could not be validated when tested for crop management and climate relations. There are few long-term studies evaluating yields, however, with Angelini et al. (2009) the only one that lasted 12 years with N fertilizer (Arundale et al., 2014b). In this study located in Italy, decreasing yields were observed from the 9^{th} to the 12^{th} year of growth (Angelini et al., 2009). In the United States, Arundale et al. (2014b) analyzed data from seven *M.* x *giganteus* sites in Illinois and found declines in yields after only six years. This was in contrast to previous studies, and was attributed to a larger nutrient removal in harvested biomass due to higher yields in the early years compared to European trials (Arundale et al., 2014b).

Determining optimum fertilization rates for *M.* x *giganteus* biomass production and understanding the C and N dynamics and seasonal changes at different stand ages is a challenge. Studies in Europe and Illinois have shown differences in yields that could be explained by climate variations and soil characteristics, as well as allocation of resources to roots and rhizomes (Dohleman et al., 2012). However, these studies in Europe and Illinois have shown the same response to N fertilizer, especially in soils with low N content (Lewandoski et al., 2000).

Behnke et al. (2012) studied the effect of three fertilization rates (0, 60 and 120 kg N ha^{-1}) on N leaching, N content and biomass yields of *M.* x *giganteus* (in Urbana, Illinois from 2008 to 2011). Their results showed no significant response of biomass yields to the fertilization treatments but the N content removed in the harvested biomass in 2010 was greater at the 60 and 120 kg N ha^{-1} fertilizer rates. There was also an increase in nitrate NO_3^- leaching between 0 and 120 kg N ha^{-1} in 2010 that was affected by the timing and amounts of precipitation events. For unfertilized *M.* x *giganteus*, Smith et al. (2013) found that the mid-profile nitrate leaching and tile nitrate concentrations were greatly reduced as a result of production.

The large reduction in N losses in surface soils and tile-drainage systems from *M.* x *giganteus* and switchgrass production compared to corn and soybean rotations has demonstrated that this perennial grass has the ability to take up mineralized N from soils (McIsaac et al., 2010; Behnke et al., 2012; Smith et al., 2013). Fertilization of *M.* x *giganteus* during the establishment period clearly increased nitrate leaching and N_2O emissions, but did not increase yields (Behnke et al., 2012, Davis et al., 2015). In addition, Davis et al. (2013a, 2015) suggested that soil organic matter can be altered by this crop, making N available from surface soil organic N pools for uptake by *M.* x *giganteus*.

Davis et al. (2013a) analyzed surface soils (0-10 cm deep) from five-year-old *M.* x *giganteus* at the Crop Science Research and Education Center located south of the University of Illinois at Urbana-Champaign. They compared N mineralization indices for these soils to traditional corn and soybean rotations in high organic matter Mollisols. Using a 7-day anaerobic incubation

(AnaN), they found significantly greater potential N mineralization in the *M.* x *giganteus* soils. Davis et al. (2013a) suggested that increasing belowground biomass of *M.* x *giganteus* enriched the soil organic matter that led to greater N mineralization rates from soil organic N pools.

The greater N mineralization could have also been attributed to high levels of microbial activity in the surface of the *M.* x *giganteus* soils. Microbes in the rhizosphere could be responsible for an acceleration in the SOM decomposition and C and N mineralization with the addition of new organic matter (Zhu and Cheng, 2013). As Bengston et al. (2012) explained, the increase in the metabolism and growth of the microbes considered r strategists can cause the emergence of secondary populations of K strategists that could ultimately increase decomposition.

In another study in Illinois, Kentucky, Nebraska, New Jersey and Virginia, biomass production, soil organic matter, and inorganic N leaching in *M.* x *giganteus* plots under different fertilization treatments where determined (Davis et al., 2015). Fertilization treatments were 0, 60, and 120 kg N ha^{-1} yr^{-1} as urea in the spring. After four years of *M.* x *giganteus* growth (2008 to 2012), the potentially mineralizable N (AnaN) of the surface soils (0-10 cm) increased under all treatments in this study. However, labile C [permanganate oxidizable C (POX-C)] increased in surface soils only in plots that previously were in row crops, whereas plots established in sod had decreases in POX-C. The response of AnaN and POX-C was not affected by N fertilizer treatment. Nitrogen fertilization did increase N_2O emissions and N leaching without increasing biomass production during the establishment phase of *M.* x *giganteus* production. Davis et al. (2015) concluded that additional research was needed to determine why potentially mineralizable N increased regardless of site history. They speculated that *M.* x *giganteus* roots and rhizomes may be playing a role.

Objectives

The overall objective of this study was to evaluate changes in soil organic matter and N losses through leaching in mature *M.* x *giganteus* stands at five sites in the United States.

Specific objectives are to:

1. determine if previous effects of N fertilization on biomass yields, leaching and soil organic C and N after the establishment phase continue through the 6^{th} and 7^{th} year of mature *M.* x *giganteus*;
2. examine labile organic matter pools to determine if microbial activity is related to potential mineralization rates in soils under *M.* x *giganteus*; and
3. monitor the effects of soil temperature and moisture on *M.* x *giganteus* growth and N leaching.

Experimental design

Field Sites

Sampling was conducted at five university field sites (Urbana, IL; Lexington, KY; Mead, NE; Adelphia, NJ; Gretna, VA) that were part of the US Department of Energy (DOE) Sungrant Program where fertilized *M.* x *giganteus* was grown. All sites were planted in 2008, with the exception of Virginia, which was planted in 2010. There were twelve 10 m x 10 m plots at each site, with four replicates of three different rates of fertilization (0, 60 and 120 kg N ha^{-1} as urea each spring). See Maughan et al. (2012) for details on site preparation and planting, Behnke et al. (2012) for previous work on the Urbana site, and Davis et al. (2015) for previous results at all locations.

The field trials at Urbana were located on the South Farms of the University of Illinois. This site has an annual precipitation of 105 cm and an annual average temperature of 10.9 °C (National Climatic Data Center, climate normal from 1981-2010). Soils are fine loamy and are classified as Wyanet series (fine-loamy, mixed, active, mesic Typic Argiudolls). It was converted to *M.* x *giganteus* in 2008 from a conventional crop system and due to a severe winter, this site had to be mostly replanted in 2009.

The Virginia site was located at the Piedmont Bioproducts Farm in Gretna, VA, owned by the Virginia Polytechnic Institute. The average for annual precipitation in this site is 115 cm and the average annual temperature is 12.6 °C. Soils are classified as the Cecil Series (fine, kaolinitic, thermic Typic Kanhapludults), Plots established in New Jersey were located at the Rutgers University Research Center in Adelphia, NJ. This area has an annual precipitation of 119 cm and an average annual temperature of 12.0 °C. Soils in this site are poorly drained and are classified as Holmdel series (fine-loamy, mixed, active, mesic, Aquic Hapludults). *M.* x *giganteus* was well established in the first year.

At University of Kentucky site, the plots were established in the Agriculture and Research Center located in Lexington, KY. This site has an annual precipitation average of 115 cm and the average annual temperature is of 13.1 °C. Soils in this site are fine silty and are classified as Maury series (fine-silty, mixed, active, mesic Typic Paleudalfs). This field was converted to *M.* x *giganteus* from Bermuda grass and had a successful establishment after the first winter.

Plots located at the University of Nebraska-Lincoln were at the Research Center in Mead, NE and have an annual precipitation of 75 cm. The average annual temperature is 9.9 °C. Soils are a Tomek series and are classified as Mollisols (fine, smectitic, mesic Pachic Argiudolls). About 21% of the plants originally planted in 2008 were killed by the severe winter and were replanted the next year.

Figure 1. Map of Illinois plots with fertilization treatments including fertilization treatments in units of kg N ha^{-1} yr^{-1}.

Figure 2. Map of Kentucky plots with fertilization treatments including fertilization treatments in units of kg N ha^{-1} yr^{-1}.

Figure 3. Map of Nebraska plots with fertilization treatments including fertilization treatments in units of kg N ha^{-1} yr^{-1}.

Figure 4. Map of New Jersey plots with fertilization treatments including fertilization treatments in units of kg N ha^{-1} yr^{-1}.

Figure 5. Map of Virginia plots with fertilization treatments including fertilization treatments in units of kg N ha^{-1} yr^{-1}.

Biomass yield sampling methods

The harvesting for biomass yield calculation was done on a yearly basis from early to late winter depending on snow cover using a standardized protocol. It was measured based on a 1 m^2 quadrant around 4 representative plants selected from each plot. These representative plants were not located at the edges of the plots and all the tillers were cut at 10 cm above the ground. In the case of Nebraska, mechanical harvesting was performed with a forage plot harvester (Cater MFG Co., Inc. Brookston, IN, USA) set to harvest 10 cm from the ground. After samples were collected, biomass was dried at 60° C for 48 hours to determine dry biomass and percent moisture at harvest.

Field Sampling for soil analysis

Initial soil samples were collected at each site (other than VA) in 2008 at planting using a 5.08 cm diameter Giddings probe. Three random cores per plot to a depth of 100 cm were collected and divided by depth (0-10, 10-20, 20-30, 30-50, and 50-100 cm), returned to the laboratory, air-dried, disaggregated, and passed through a 2 mm sieve. Subsamples were composited by depth within each of the 12 plots at each site. Davis et al. (2015) collected nine soil cores (using a 3.2 cm diameter hand corer) from each plot, and divided them into 0-10 and 10-30 cm depths. Again, samples were air-dried, disaggregated, and passed through a 2 mm sieve. The 10-20 and 20-30 cm samples from 2008 were composited to match the 10-30 cm samples collected in 2012

(Davis et al., 2015). In the spring of 2014, soil samples were again collected using the Giddings probe at each site following the 2008 protocol.

For nitrate and ammonium leaching in the soils, resin lysimeters following the design of Susfalk and Johnson (2002) were used. These have previously been used by McIsaac et al. (2010), Behnke et al. (2012), Smith et al. (2013), and Davis et al. (2015). These lysimeters have a cation/anion exchange resin layer inside of a 5.1 cm diameter PVC pipe and two layers of sand. The bottom layer has a 1 cm layer, whereas the top layer has 5 cm of sand to avoid soil contamination through direct contact with the resin and for hydrologic contact. In Illinois, resin lysimeters were installed in 2009 and at the other states in 2012 at a depth of 50 cm. Two 60 cm holes were excavated in each plot and inside of each hole two resin lysimeters were collocated in opposite directions. Lysimeters were placed in the soil profile so that the soil above them was undisturbed. The holes were then filled and returned to their original position. Lysimeters were left in the soils for 1 year and then replaced with new ones. Resins were processed in the laboratory after collection and extractable inorganic N determined (see below).

Analytical procedures

Soil samples collected in spring 2014 to a depth of 1 m were sent to an outside laboratory for the same full analysis as the 2008 samples. The 2008 values were reported in Maughan et al. (2012), and included pH, CEC, organic matter, total C and N, C:N ratio, extractable P, K, Ca, Mg, and S.

Resin lysimeters were analyzed for extractable inorganic N (nitrate and ammonium) with a 40 ml of 2 M potassium chloride following Mulvaney (1996). The extracts were analyzed for NH_4-N and NO_3-N on a Lachat Quikchem FIA 8000 series Flow Injection Analysis System. Soil samples collected in 2014 were analyzed for KCl extractable nitrate and ammonium.

For potential mineralizable N (AnaN), the 7-day anaerobic incubation technique was used. Approximately 8 g of soil with 10 mL of distilled deionized water were put into a 50 mL centrifuge tube and then purged with N_2 gas for one minute. The centrifuge tubes were sealed with electrical tape around the cap edges. Samples were incubated at 40° C for 7 days. The samples were removed from the incubator and 30 mL of 2.67 M KCL was added to each tube and shaken for 1 hour, then centrifuged and extracted. Total mineralized N was calculated by subtracting the KCl NH_4-N from the NH_4-N after the incubation.

To measure the labile carbon pools permanganate oxidizable carbon (POX-C) was determined. The protocol for this procedure follows Culman et al. (2012). For each sample, 2.5 g were placed into a centrifuge tube and one soil sample were designated as a quality control. Samples were covered with 2 mL of 0.2 M $KMnO_4$ and 18 mL of distilled deionized water. Samples then were immediately shaken for 2 minutes and then allowed to settle for 10 minutes in the dark. The samples then were diluted by extracting 0.5 mL of the supernatant and mixed with 49.5 mL of distilled deionized water. After this, samples were analyzed using a spectrophotometer (Aquamate, Thermo Spectronic) with as low of light as possible. The absorbance values were used to calculate POX-C. Triplicates of $KMnO_4$ stock solution at concentrations of 0.005, 0.01, 0.015, 0.02 M were used to calculate the standard curve for the following equation by Weil et al. (2003):

POX-C (mg kg $^{-1}$ soil) = [0.02 mol L^{-1}-(a+b×Abs)×(9000 mg C mol^{-1}) × (0.02 L solution wt^{-1})

Where:

0.02 mol L^{-1}= initial concentration

a= intercept of the standard curve

b= slope of the standard curve

Abs= Absorbance of unknown simple

9000= milligrams of carbon dioxide by 1 mole of MnO_4 changing from $Mn^{7+} \rightarrow Mn^{4+}$

0.02 L= volume of stock solution reacted

wt= weight of air dried soil in kg

The microbial activity was measured through the fluorescein diacetate (FDA) hydrolysis assay to measure relative enzyme activity. For this procedure 1 g of soil was mixed with FDA and a phosphate buffer solution and incubated and shaken for 5 hours at room temperature. The reaction is later stopped by adding acetone and letting it stand for 10 min. The results of this reaction produces a yellow-green color that indicates the amount of enzymes presents and the intensity of the color is quantified by spectrophotometry at a wavelength of 490 nm. The amount of fluorescein produced by the sample was obtained by subtracting no FDA controls.

Statistical analysis

Statistical Analyses were performed using SAS v9.2 (SAS Institute Inc., 2011). Soil measurements were compared for significant difference using a PROC MIXED model with lsmean test for paired comparisons. The fixed variables used in this model were site, year, fertilization treatment and depth and replicates were used as a random variable. Significance between treatments was measured at $\alpha = 0.05$.

Soil temperature and moisture monitoring

Soils were monitored for temperature at 10 cm and for moisture with Em5b data loggers (Decagon Devices) and 10HS moisture sensors at 10, 30 and 50 cm depths were installed at each site and recorded measurements on an hourly basis.

Chapter 2: Results and Discussion

Biomass Yields and Fertilization

Harvested biomass yields of *M.* x *giganteus* on this study increased in 4 out of 5 sites compared to the first year after the establishment (Figure 13). This tendency has not been consistent over years and some sites had low yield years that might be related to climatic conditions, such as a lack of soil water. During the past three years (2013-2015) two sites have shown declining yields (Illinois and Kentucky), whereas three sites had increases in their productivity (Nebraska, New Jersey, and Virginia).

Nebraska and New Jersey (7 years after planting) along with Virginia on its 5th year of production had their largest harvested biomass yields in 2015. On the other hand, in Illinois and Kentucky productivity had a declining trend in yields from the maximum achieved in 2013, but yields were still higher than the first two years of production.

In Illinois, biomass yield increased consistently during the 5 years of the study with the exception of 2012 when average yields went down to 10 Mg ha^{-1}; however, 2013 was the year with highest yields overall when fertilized. A possible explanation for the 2012 low yields is that this site suffered a severe drought in 2012 that lasted several years with almost 300 mm less precipitation than the 1009 mm thirty-year normal. Starting in 2011 and going through 2015, plots with any fertilizer treatment (60 or 120 kg N ha^{-1}) had harvested biomass yields that were significantly greater than unfertilized plots. However, differences between 60 and 120 kg N ha^{-1} fertilization treatments were not observed in any year.

Kentucky's biomass yields had a less clear tendency over the years. On this site the first harvest was higher than any other state and they kept going up and down for the next 5 years with 2015 year being one of the lowest. In 2010 and 2012 there was less precipitation than the 30 year normal, and their yields went down with recovery in 2011 and 2013, respectively. Over 7 years, 2010 was the driest year which might have harmed the crop and had an influence on the 2010 and 2011 harvests. These two years had significant differences between the fertilizer treatments. However, in 2010 none of the fertilized yields were significantly different than the unfertilized plots and in 2011 the harvested biomass in plots without fertilizer were actually greater than the fertilized plots. A possible explanation for this might be related to large precipitation events that occurred in the months of April and May after the application of fertilizer and may have led to large losses.

Biomass yields in Nebraska were greater than all other sites and Nebraska is historically the driest site from all locations but with the most productive soils in terms of dry biomass. Harvested biomass yields reached a peak in 2015 with 120 kg N ha^{-1} and the second greatest was also with 120 kg N ha^{-1} in 2011. Yields have stayed greater than the establishment year for the 6 years and 2015 unfertilized plots had very high yields reaching almost 30 Mg ha^{-1}. Nebraska was also severely affected by the 2012 drought with only 468 mm of rain compared to the 748 mm year normal, which might have been the cause of the decrease in yields after the productive 2011. It was not until 2015 that the crop yields recovered completely and the effect of supplemental N was observed.

New Jersey's biomass yields have increased compared to the first year with three years that had declines in yields and that were also characterized by lower precipitation levels than average (2010, 2012 and 2013), but with a hurricane in 2013 that affected the crop during the growing season. During this year, unfertilized plots had poor yields but in 2014 and 2015 the production improved with the highest production when plots were fertilized. In 2015, plots that had 60 kg N ha^{-1} had the highest yields however, plots with 120 kg N ha^{-1} leached the greater amount of ni-trate.

Virginia had only 5 years of yield data, since it was planted in 2010. Since then the average yield of all treatments has increased and since the 4^{th} year there has been a significant difference in bi-omass yields between fertilized and unfertilized plots. The unfertilized plots yields have de-creased after the 2nd year and have been decreasing since then. Fertilized plots starting in 2013 have had higher yields and in 2014 and 2015 even though fertilized plots had higher years than unfertilized, there is no significant difference in productivity between 60 and 120 kg N ha^{-1} but this might indicate that after the establishing period the addition of some N has been necessary.

M. x *giganteus* have been reported to be highly productive in the past. Studies have shown as much as 37 t ha^{-1} yr^{-1} yields with irrigation and fertilization of 200 kg N ha^{-1} in the third year af-ter planting (Ercoli et al., 1999) and in mature stands as much as 17.7 t ha^{-1} in the 10th year (Christian et al., 2008). In a study in England on silty clay loam soils in 1993 no effect of N ferti-lizer was found during their 15-year study (Christian et al., 2008). *M.* x *giganteus* plantations with appropriate growing conditions have not been shown to require the application of N fertilizer during the establishment phase since it does not produce increases in yields. However, after the 5^{th} and 6^{th} year of production, this study showed an overall significant effect of N fertilization on biomass yields (Table 1 and 2.) which indicates that when *M.* x *giganteus* reaches a mature phase or when extreme weather conditions have affected it, N fertilization seems to improve biomass production.

Table 1.Results (P values) of the effects of the fixed variables of the PROC MIXED model.

Effect	Potential Mineralization N	Labile C	Microbial Activity	Total C	Total N	NO3 leaching	NH4 leaching	Biomas Yield
Fert	0.42	0.97	<0.0001	0.92	0.9	<0.0001	<0.0001	<0.0001
Year	<0.0001	<0.0001	-	0.0009	0.01	0.0013	<0.0001	<0.0001
Site	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Depth	<0.0001	<0.0001	-	<0.0001	<0.0001	-	-	-
Fert-Year	0.93	0.6	-	0.94	0.97	0.0519	<0.0001	<0.0001
Fert-Site	0.69	0.64	<0.0001	0.346	0.92	<0.0001	<0.0001	<0.0001
Fert-depth	0.5	0.45	-	0.325	0.588	-	-	-
year-site	<0.0001	0.0056	-	0.009	0.0002	<0.0001	<0.0001	<0.0001
year-depth	<0.0001	0.054	-	<0.0001	<0.0001	-	-	-
site-depth	<0.0001	<0.0001	-	<0.0001	<0.0001	-	-	-
site-fert-year	0.97	0.73	-	0.1179	0.69	0.0006	<0.0001	0.0026
year-site-depth	<0.0001	<0.0001	-	<0.0001	<0.0001	-	-	-
site-depth-fert	0.56	0.663	-	0.319	0.817	-	-	-
depth-fert-year	0.94	0.663	-	0.09	0.519	-	-	-
site-depth-year-fert	0.72	0.36	-	0.245	0.785	-	-	-

Table 2. Means and standard error of the effect of fertilization treatment and site on biomass production (Mg ha^{-1}).

	Biomass	
Fertilization	Mean	SE
0	15.60	6.43
60	18.10	6.50
120	18.69	7.23
Site		
IL	16.11	8.48
KY	15.56	3.58
NE	23.39	6.94
NJ	16.05	5.33
VA	15.72	4.85

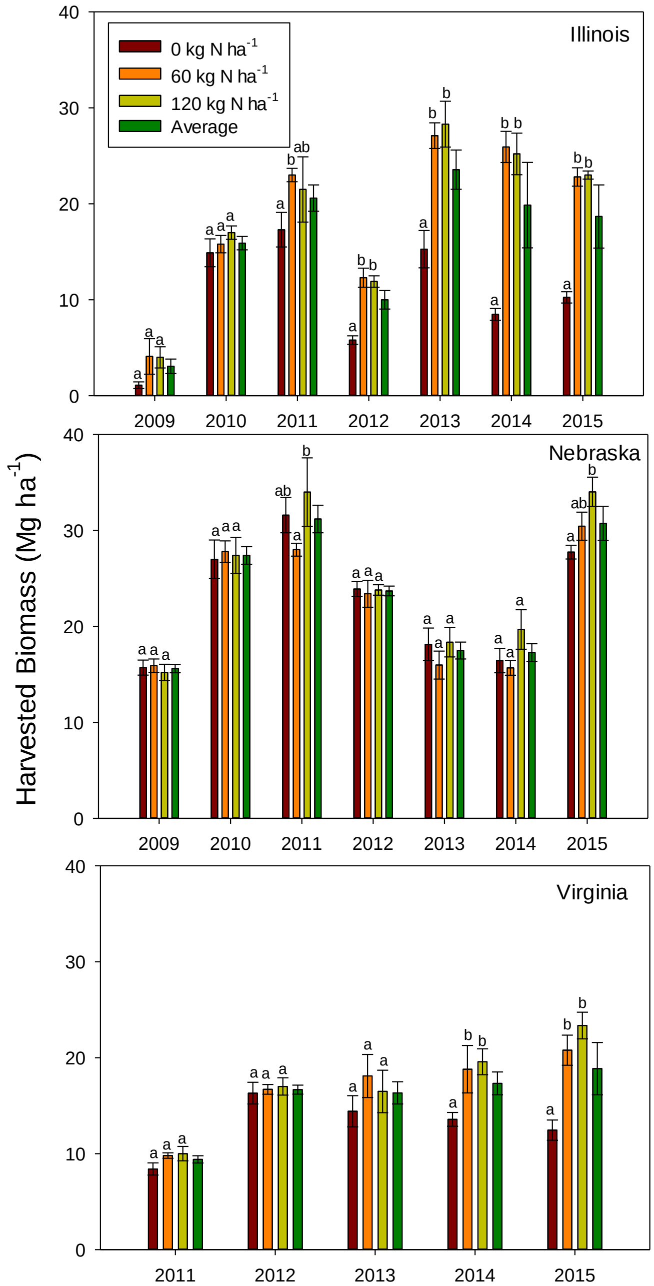


Figure 6. Harvested biomass yields (Mg ha^{-1}) from 2009 to 2015 in Kentucky and New Jersey. Standard errors are shown. Letters represent a significant difference between fertilization treatments.

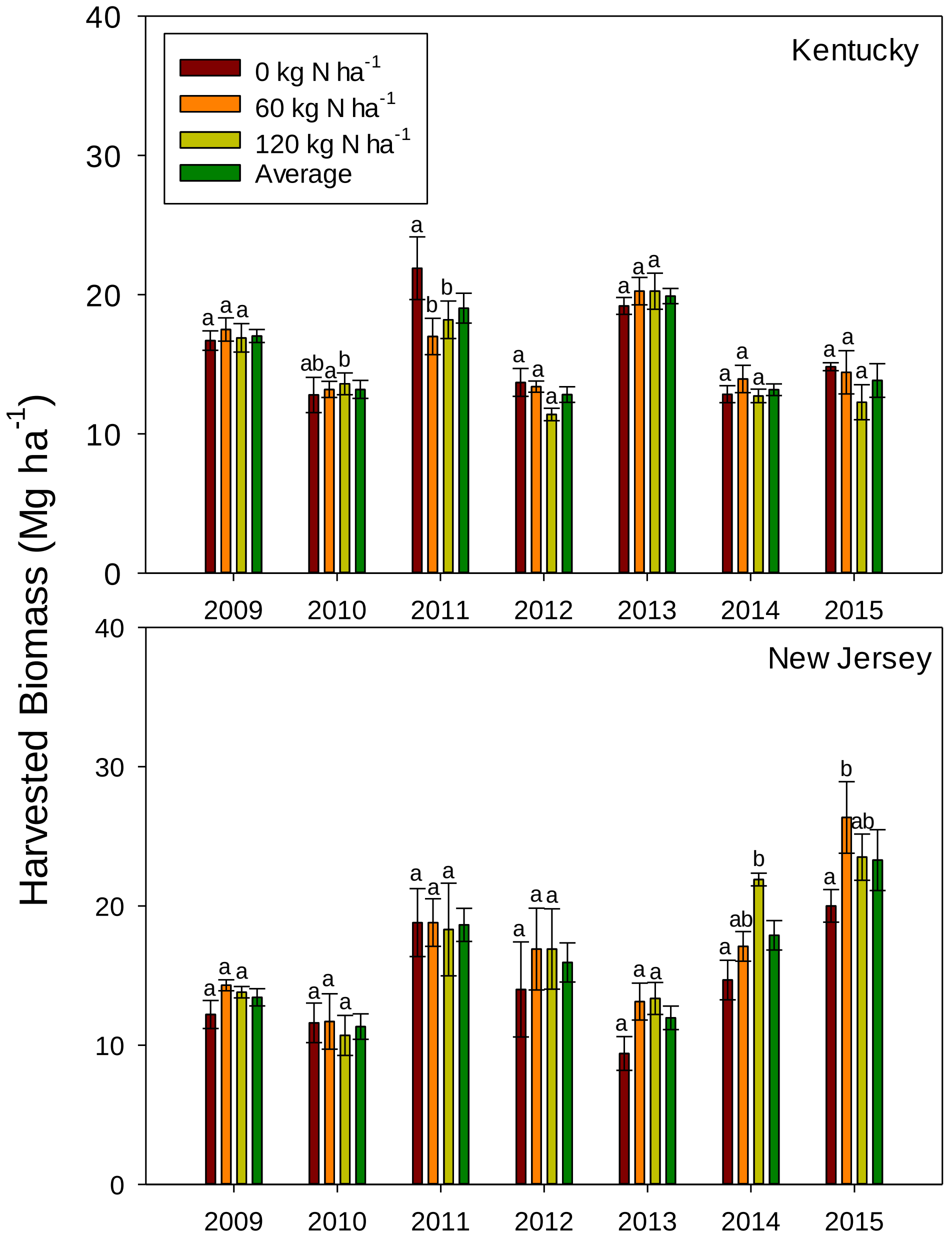


Figure 7. Harvested biomass yields (Mg ha^{-1}) from 2009 to 2015 in Kentucky and New Jersey. Standard errors are shown. Letters represent a significant difference between fertilization treatment.

Inorganic N Leaching

Nitrate leaching clearly responded to fertilizer treatments during the 2013 and 2014 growing years. The resin lysimeters collected during the spring of 2014 and 2015 for the 2013 and 2014 growing years had significantly greater nitrate leaching in fertilized plots at most sites, with the greatest nitrate leaching in plots fertilized with 120 kg N ha^{-1}.

Nitrate leaching in unfertilized plots ranged from 0.4 kg N ha^{-1} yr^{-1} in New Jersey (2015) to 10.2 kg N ha^{-1} yr^{-1} in Kentucky (2014). In plots fertilized with 60 kg N ha^{-1}, the range in nitrate leaching was from 1.5 kg N ha^{-1} yr^{-1} in Virginia (2015) to 13.8 kg N ha^{-1} yr^{-1} in Kentucky (2014) and in plots fertilized with 120 kg N ha^{-1}, from 6.5 kg N ha^{-1} yr^{-1} in Virginia (2015) to 35.9 kg N ha^{-1} yr^{-1} in Kentucky (2014).

Overall, nitrate leaching varied across sites during the 6th and 7th growing seasons. However, in Illinois, we found an expected nitrate leaching behavior for *M. x giganteus*. Behnke et al. (2012) and Davis et al. (2015) suggested that nitrate leaching declined after the first years of *M. x giganteus* establishment and that was again supported by this study. Lesur et al. (2014) also suggested that the age is a determining factor in the higher nitrate leaching in the first years after establishment and that might be the ref lection of the low development of roots. In Illinois, after the second year of *M. x giganteus* where the nitrate leaching reached a peak of 35.1 kg N ha^{-1} yr^{-1} in 120 kg N ha^{-1} plots the amount of N loss decreased and in 2013 year growing season it was almost as low as the first year with only 18.9 kg N ha^{-1} yr^{-1}. In 2014 however, nitrate leaching losses increased to 22.8 kg N ha^{-1} yr^{-1}. This is also true for plots fertilized with 60 kg N ha^{-1} and unfertilized plots where losses were even lower, 0.9 and 4. 6 kg N ha^{-1} yr^{-1}, than the establishment year, 7.1 and 7.2 kg N ha^{-1} yr^{-1}, respectively.

The common pattern at all sites in nitrate leaching in relation to fertilizer was also observed by Behnke et al. (2012) and Davis et al. (2015). The plots fertilized with 120 kg N ha^{-1} had the greatest nitrate leaching and at all sites nitrate leaching increased as fertilizer inputs increased. The exception was Virginia (2014), where there was less leaching in the 60 kg N ha^{-1} treatment than the unfertilized treatment. However, the nitrate loss for the 60 kg N ha^{-1} treatment was not significantly different than the unfertilized plot, but both were significantly less than losses in the 120 kg N ha^{-1} treatment. This pattern in Virginia was also observed in the 2012 growing year and it might have been related to small differences in soil topography.

On average, the sites with less nitrate leaching overall were New Jersey and Virginia and the higher nitrate leaching was observed in Kentucky and Nebraska. These two sites had considerably higher nitrate leaching in 2013 and 2014 which can be related to large precipitation events during those years but data from 2015 were extremely high and were not used for comparisons. However, I observed a difference in the Kentucky plots because of their geographical location with half of the plots located on the summit of the landscape and half on a back of the slope. Plots located on the summit of slope had significantly greater nitrate leaching in 2012 and 2013 growing years. A possible cause for this difference might be a change in elevation that would allow more water to leach in the area with the lower water table, a trend that was also observed by Davis et al. (2015).

Large precipitation events can lead to an increase in N leaching, and might explain the high nitrate losses from Kentucky during 2015. Kentucky plots are divided spatially in two different locations; however, the extreme high nitrate results were found in both of them in 2015. March and April 2015 were particularly wet months in Lexington, Kentucky with 189 and 290 mm of precipitation compared to the 30 year normal of 91 and 134 mm (National Climatic Data Center, climate normal from 1981-2010). Also, there was a precipitation event less than a month before the collection of resin lysimeters installed in Kentucky and the heavy rainfall could have increased the water table enough so that lysimeters could have been contaminated from ground water and not only from leaching through the soil profile. The results in this site were consistent through all the fertilizer treatments and in both locations of the Kentucky plots. In addition, samples from all sites were analyzed at the same time in the same laboratory and laboratory blanks were run in the middle of the analysis to be sure there was not contamination of the samples during the extraction of the resin. I have no explanation for the extremely large nitrate leaching values measured in Nebraska in 2015, and because they were not believable they were removed and not further analyzed.

I found an increase in ammonium leaching over the years in all sites with the exception of Virginia. However, only the Illinois and New Jersey site had significant difference between the fertilizer treatments with increased ammonium leaching with larger fertilizer treatments. In Illinois, the increase started after the third year and in 2013 the 120 kg N ha^{-1} of fertilizer had increased ammonium leaching to 24 kg N ha^{-1} yr^{-1}, it decreased to 18 kg N ha^{-1} yr^{-1} in 2014 but remained significantly higher than the establishment year (2.7 kg N ha^{-1} yr^{-1}). Davis et al. (2015) attributed the higher leaching of ammonium in Illinois to sandy texture of the soil in this site and the low cation exchange capacity that is characteristic of sandy soils.

Leaching of total inorganic N happens mainly in the form of nitrate that moves easily along with water in soils, with only a small amount of the total inorganic N being from ammonium leaching that tends to held to clay particles due to its positive charge. Fertilizer treatments, soil texture and precipitation would be expected to have an influence in the amount of inorganic N leaching. *M. x giganteus* soils are expected to have less leaching than corn and soybean plantation with an average of 3 kg N ha^{-1} yr^{-1} (McIsaac et al., 2010). In this study, nitrate and ammonium leaching were proved to be affected by fertilization rate and varied from site to site however (Table 3.), the soil texture and precipitation effect need to be evaluated in future studies.

Table 3. Means and standard error of the effect of fertilization treatment and site on nitrate and ammonium leaching kg N ha^{-1} yr^{-1}.

	NO_3		NH_4	
Fertilization	Mean	SE	Mean	SE
0	2.93	5.86	3.69	3.17
60	9.13	11.10	4.96	5.52
120	20.61	20.16	6.93	8.68
Site				
IL	12.37	14.43	8.44	8.38
KY	12.24	18.52	2.55	2.11
NE	18.66	25.29	2.66	4.32
NJ	8.45	10.04	5.44	4.27
VA	4.65	6.44	1.83	0.73

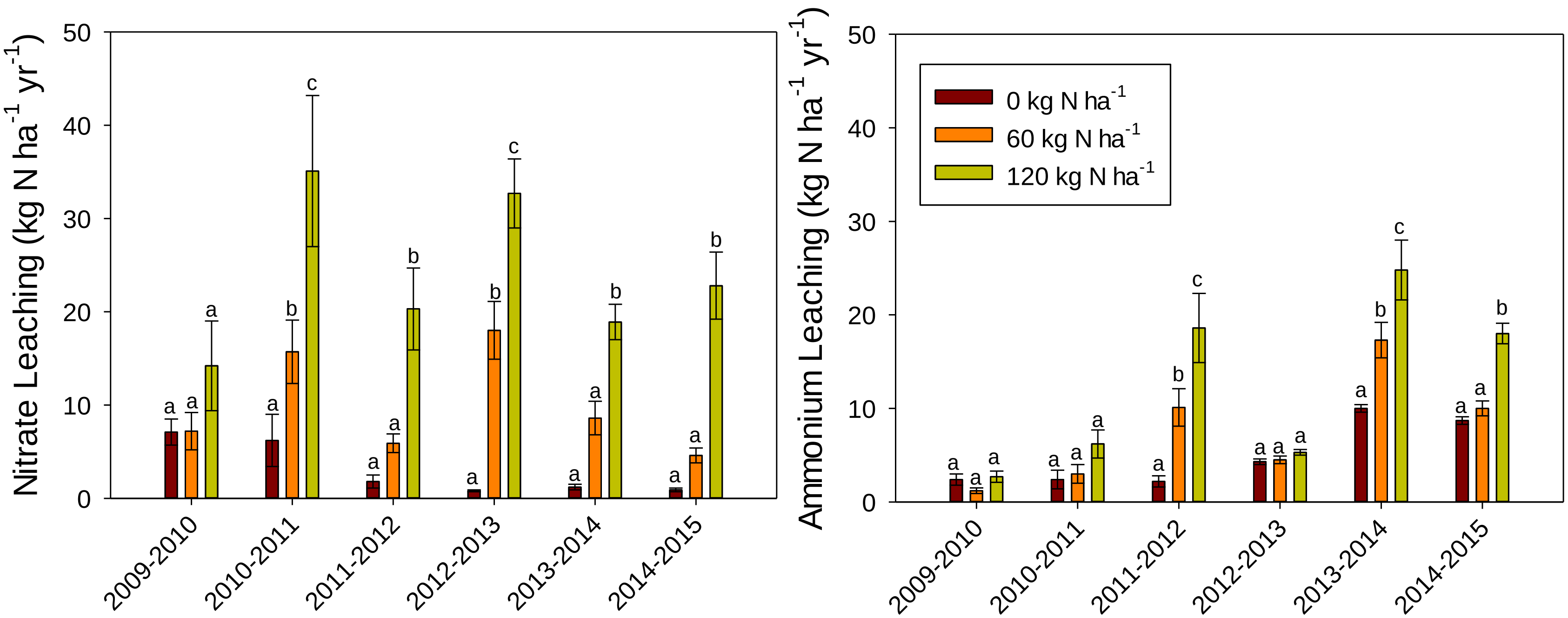


Figure 8. Nitrate and ammonium leaching in Illinois by fertilizer rate in Illinois from 2009 to 2015 with standard errors. Letters indicate significant difference between treatments.

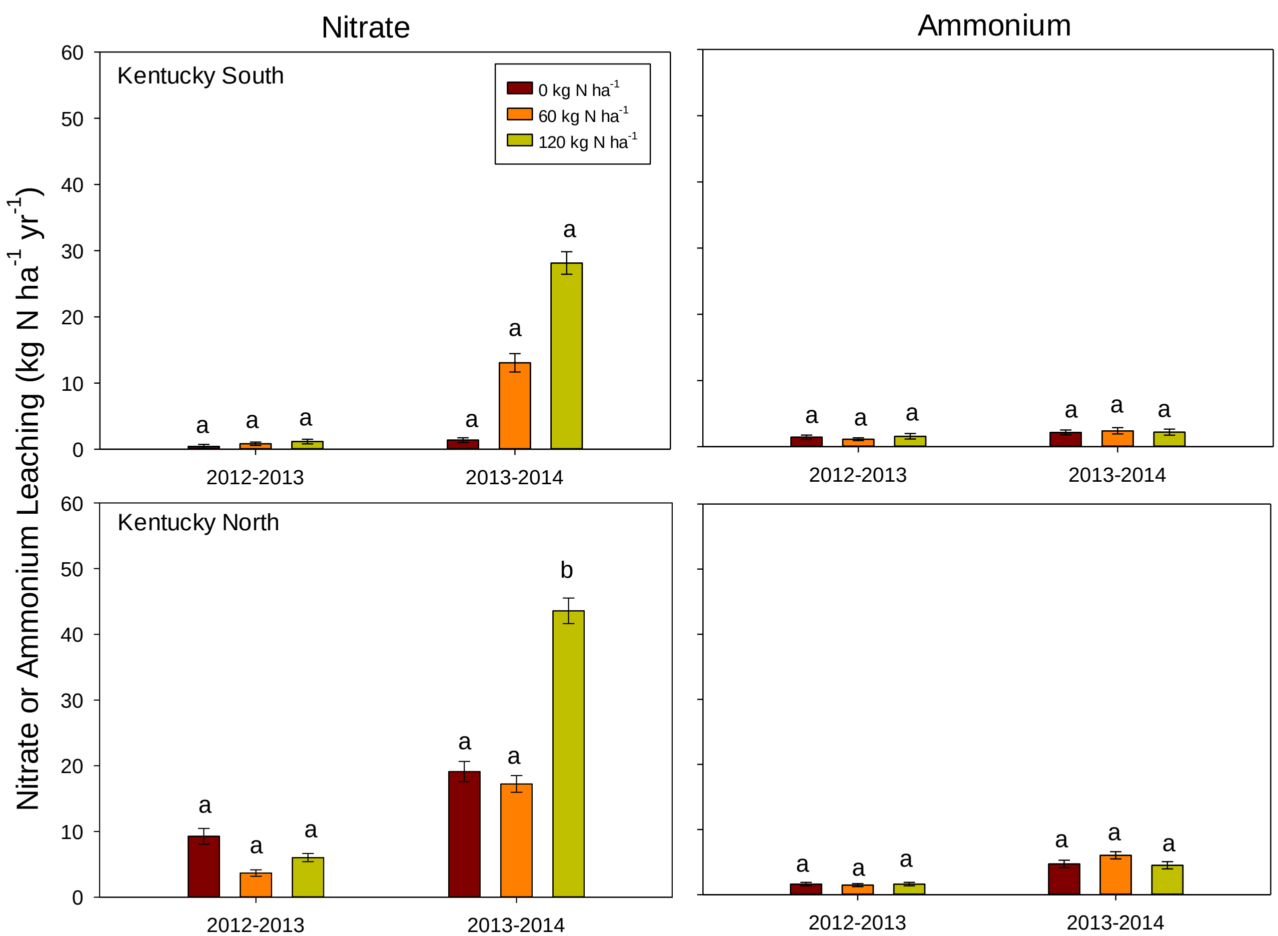


Figure 9. Nitrate and ammonium leaching in Kentucky by fertilizer rate from 2012 to 2014 with standard errors. Letters indicate significant difference between treatments.

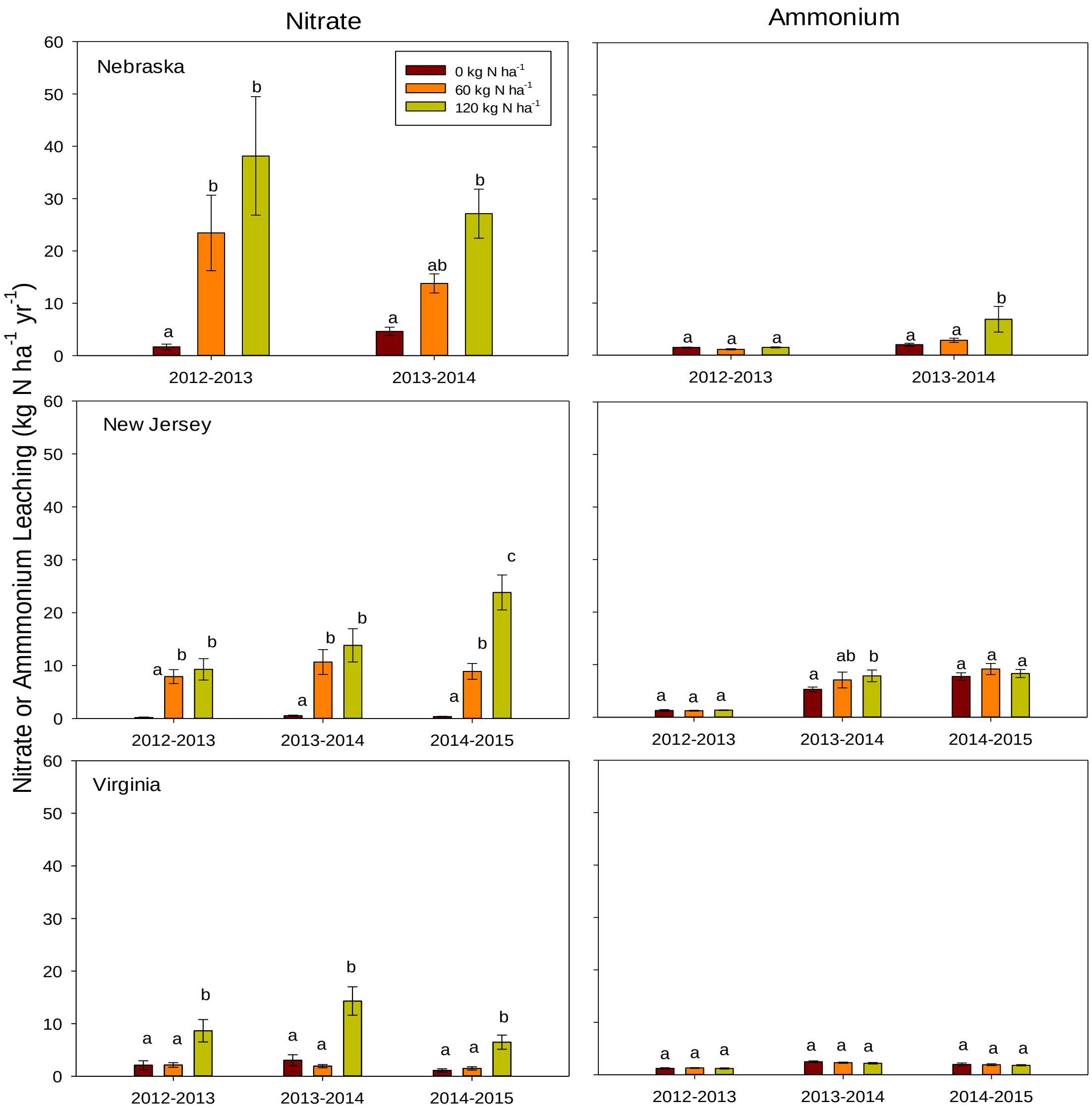


Figure 10. Nitrate and ammonium leaching in Nebraska, New Jersey, and Virginia by fertilizer rate from 2012 to 2015 with standard errors. Letters indicate significant difference between treatments.

Soil Total Carbon and Nitrogen

Across the five sites in this study, I observed a significant decrease in total C and N during the study period. Changes in C and N were not observed on the 10-30 cm soil depth with the exception of total N in Kentucky from 2012 to 2014 where it increased from 0.16% in 2008 to 0.18% in 2014 (Table 2).

Kentucky and Nebraska would be expected to showing similar decreasing tendencies in labile C and Illinois and New Jersey were expected to show an increase well since they have a similar soil history. Soil labile C measured with POX-C was greater in Nebraska and Kentucky compared to other states at 0-10 cm soil depth and Illinois had the smallest labile C as well as total C. A significant difference was found only at Illinois, where the labile C was less than in the 2012 samples, and New Jersey in which the labile C has been increasing since the establishment of the crop. For the 10-30 cm soil depth there were even less changes and the only important change was observed in Nebraska that had the smallest amount of labile C since the establishment of the crop, and Virginia where there was a decrease from 2012 to 2014 (Table 3).

Perennial grasses can increase soil C and N sequestration (Al-Kaisi et al., 2005) and improve conditions for decomposers like soil moisture and increase organic matter and soil aeration from root biomass and according to a modeling study by Davis et al. (2010) of soils under *M. x giganteus* found they have the potential to accumulate total C due to the large above ground biomass and root production. However, after 8 years of N fertilization there were no significant changes in soil total N or C across fertilization treatments at any depth. Studies have shown that high rates of N fertilization can increase the total N and C in surface soils (Raun et al., 1998; Blevins et al. 1983; McAndrew and Malhi 1992), but this was not observed in this study.

Changes in POX-C were not clearly related to previous land use. Sites that were previously in row crops, corn or soybean with conventional tillage had greater concentrations of labile C in 2012 than the establishment year however, the increase shown in previous studies in Illinois (Davis et al. 2015) was not observed in 2014 in this study even though New Jersey did show a significant increase. Changes in sites that were previously in turfgrass did not show a response in POX-C concentrations as was observed in 2012.

The labile portion of the total C pool is also an indicator of short-term changes in soils and it was measured through POX-C. It is expected to be responsive to the land conversion to *M. x giganteus* and to management practices because it tends to be sensitive to environmental variations like climatic variations and soil type (Culman et al., 2012). In this study, changes that were observed in labile C were only for the upper layer of the soil (0 – 10 cm). This might be a result of changes in soil conditions in the layer with higher root density after previous land management and the changes in soil organic matter inputs from *M. x giganteus* as well as soil disturbance. This might also be related to microbial biomass in soils since POX-C measurements were consistent with microbial activity in 2014; sites with higher microbial biomass had higher measured active C. Fertilization rates had no significant effect on changes either in total C and N, nor in labile carbon in this study.

Table 4. Means and standard error of the effect of fertilization treatment and site on total C and N (%).

	Total C 0-10 cm		Total C 10-30 cm		Total N 0-10 cm		Total N 10-30 cm	
Fertilization	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	1.75	0.64	1.24	0.38	0.17	0.07	0.13	0.04
60	1.67	0.74	1.29	0.43	0.17	0.08	0.14	0.04
120	1.74	0.72	1.24	0.40	0.17	0.08	0.13	0.04
Site								
IL	1.07	0.22	1.12	0.38	0.11	0.02	0.11	0.02
KY	2.08	0.68	1.36	0.25	0.23	0.07	0.17	0.02
NE	2.62	0.22	1.72	0.19	0.26	0.02	0.18	0.02
NJ	1.37	0.34	1.18	0.26	0.12	0.03	0.11	0.03
VA	1.31	0.21	0.73	0.10	0.12	0.02	0.07	0.01

Table 5. Total C and N at 0-10 and 10-30 cm soil depths from all sites. Letters indicate significance different of means within a site and depth (α=0.05).

		Total C (%)		Total N (%)	
Location	Year	0-10cm	10-30cm	0-10cm	10-30cm
Illinois	2008	1.05 ab	1.12 a	0.11 a	0.11 a
	2012	1.21 a	1.08 a	0.11 a	0.11 a
	2014	0.95 b	1.17 a	0.10 a	0.11 a
Kentucky	2008	2.48 a	1.27 a	0.28 a	0.16 a
	2012	2.25 a	1.31 ab	0.24 b	0.17 a
	2014	1.52 b	1.52 b	0.17 c	0.18 b
Nebraska	2008	2.79 a	1.69 a	0.27 a	0.18 a
	2012	2.5 b	1.78 a	0.25 a	0.17 a
	2014	2.57 ab	1.71 a	0.26 a	0.18 a
New Jersey	2008	1.22 a	1.15 a	0.12 a	0.11 a
	2012	1.54 b	1.28 a	0.13 a	0.12 a
	2014	1.34 ab	1.10 a	0.12 a	0.11 a
Virginia	2012	1.43 a	0.74 a	0.12 a	0.07 a
	2014	1.20 a	0.71 a	0.11 a	0.07 a

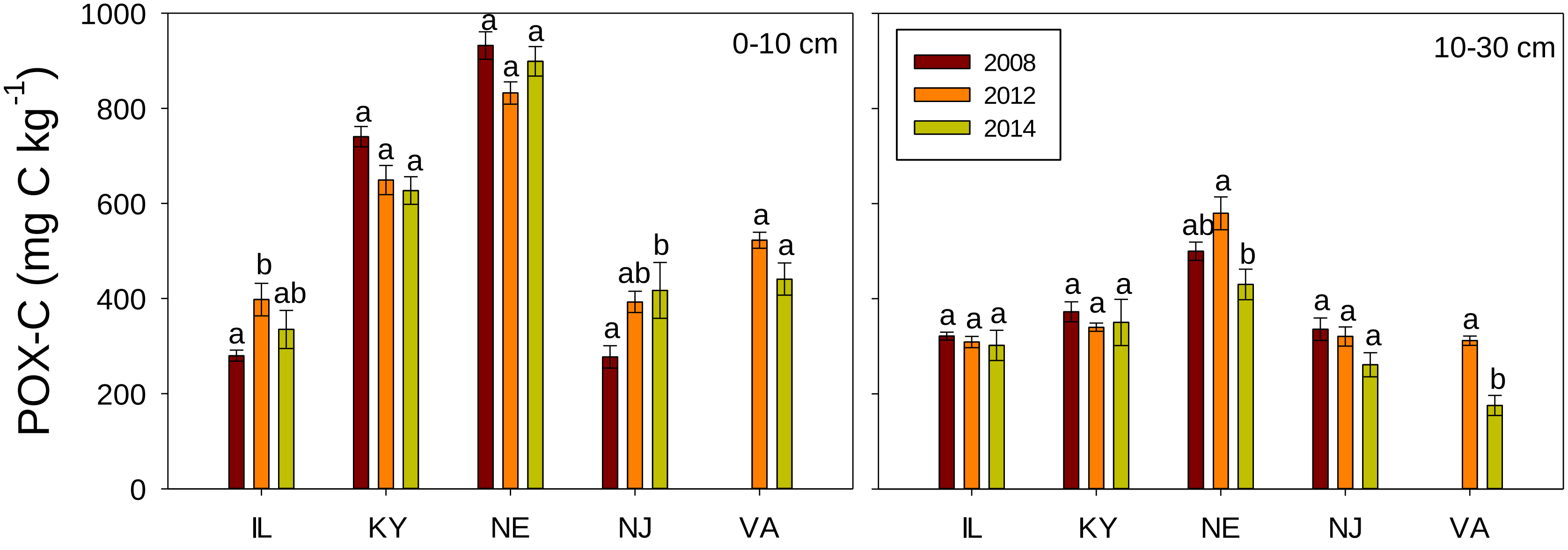


Figure 11. POX-C at 0-10 and 10-30 cm soil depths. Error bars represent the standard error. Letters indicate significance different of means between years

Soil Nitrogen Mineralization

Potential mineralization of N in the 0-10 cm soil layer in 2014 ranged from 25 mg N kg^{-1} in Illinois to 70.9 mg N kg^{-1} in Nebraska and in the 10-30 cm layer it ranged from 4.9 mg N kg^{-1} in Virginia to 37.6 mg N kg^{-1} in Nebraska.

Nebraska follow the same trend as in previous studies and is the only site with a significant increase in potentially mineralizable N reaching 70.9 mg N kg^{-1} in 2014. However, for Virginia, there was a significant decrease in mineralizable N from 37.9 to 25.3 mg N kg^{-1} from 2012 to 2014. The harvesting technique might impact the rate at which mineralization occurs due to the lack of organic resides added to the surface soils each year but since every site was harvested in the same way, it is not very likely that changes are related to harvest methodology in this study.

Previous research by Davis et al. (2015) on these sites showed that changes in potential mineralization of N did not occur in response to fertilizer treatments and sites that have previously been on a corn-soybean rotation would be expected to have an increase in potential N mineralization. In this study, Nebraska and Kentucky sites were previously turfgrass plantations, while the rest of the sites were conventional row crops. Potential N mineralization measured in 2014 did not result in consistent increases at all sites with all fertilizer treatments. Soils from the 0-10 cm depth had greater potential mineralization of N than in the10-30 cm depth, where all sites had a gradual decrease since the establishment of the crop.

A possible acceleration on the decomposition rate of SOM due to the addition of fresh organic matter could be measured with the microbial activities in soils. FDA results showed a correlation between the microbial activity in each site and the potential mineralization of N in 2014. The correlation coefficient of 0.84 suggests that the higher enzymatic activity in soils the higher N mineralization potential the soils have, although this relationship was driven mainly by one site. Microbial activity was also consistent with POX-C. Soils with higher FDA results also had the highest amounts of labile C.

Although other factors affect N mineralization rates, Nebraska's high N mineralization potential and labile C results might be related to the abundant microorganisms in this soils and the rate at which they decompose residues, and the opposite can be said in Illinois, in which the three analyses had the lowest results out of all sites for the 0-10 cm soil layer. To consider a priming effect that might be attributed to the production of *M. x giganteus* FDA analysis over the years would be recommended to determine if there is an increase in microbial activity and if this is producing an increase in decomposition of organic matter.

In mature *M. x giganteus*, enzymatic activity was significantly affected by fertilizer treatments (Table 1.) However, the significant difference in microbial biomass in these plots did not represent a change in potential mineralization of N and active C (Table 4.). It is possible that in the long term, the effect of fertilizer changes microbial populations and that this would be reflected in increases in labile C and N mineralization but further studies might be needed.

Table 6. Means and standard error of the effect of fertilization treatment and site on AnaN, POX-C and FDA.

	AnaN mg N kg^{-1} 0-10 cm		AnaN mg N kg^{-1} 10-30 cm		PoxC mg C kg^{-1} 0-10 cm		PoxC mg C kg^{-1} 10-30 cm		FDA	
Fer-tilization	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	34.60	18.41	27.26	14.06	557.40	299.47	341.49	123.27	21.75	15.80
60	33.17	19.37	27.75	13.96	558.99	189.00	360.90	106.39	26.70	23.29
120	34.54	18.37	29.03	14.85	543.75	243.43	349.72	150.79	24.07	20.12
Site										
IL	24.58	4.29	21.94	6.74	337.54	115.65	310.77	68.50	9.33	3.39
KY	34.58	21.64	25.10	7.31	672.21	104.68	354.56	106.42	23.32	4.31
NE	53.51	18.48	47.71	10.70	887.83	103.07	503.05	116.70	61.61	11.95
NJ	25.43	14.01	28.26	9.28	362.41	144.08	305.88	84.26	11.78	2.28
VA	31.56	10.17	11.58	7.32	481.68	99.70	243.74	88.95	17.75	4.46

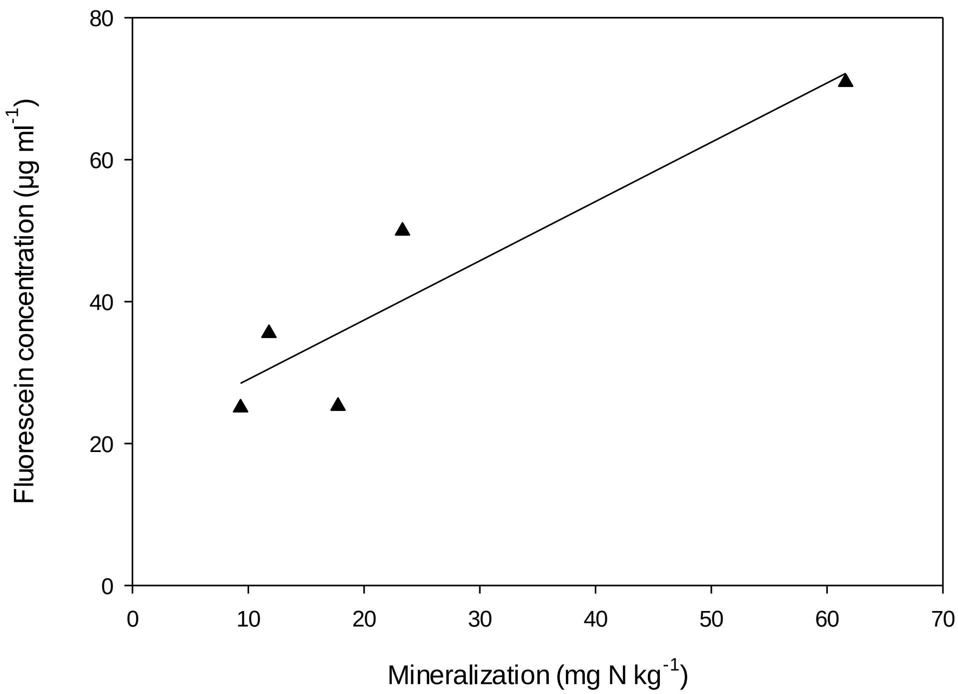


Figure 12. Relationship between potential N mineralization and fluorescein concentration.

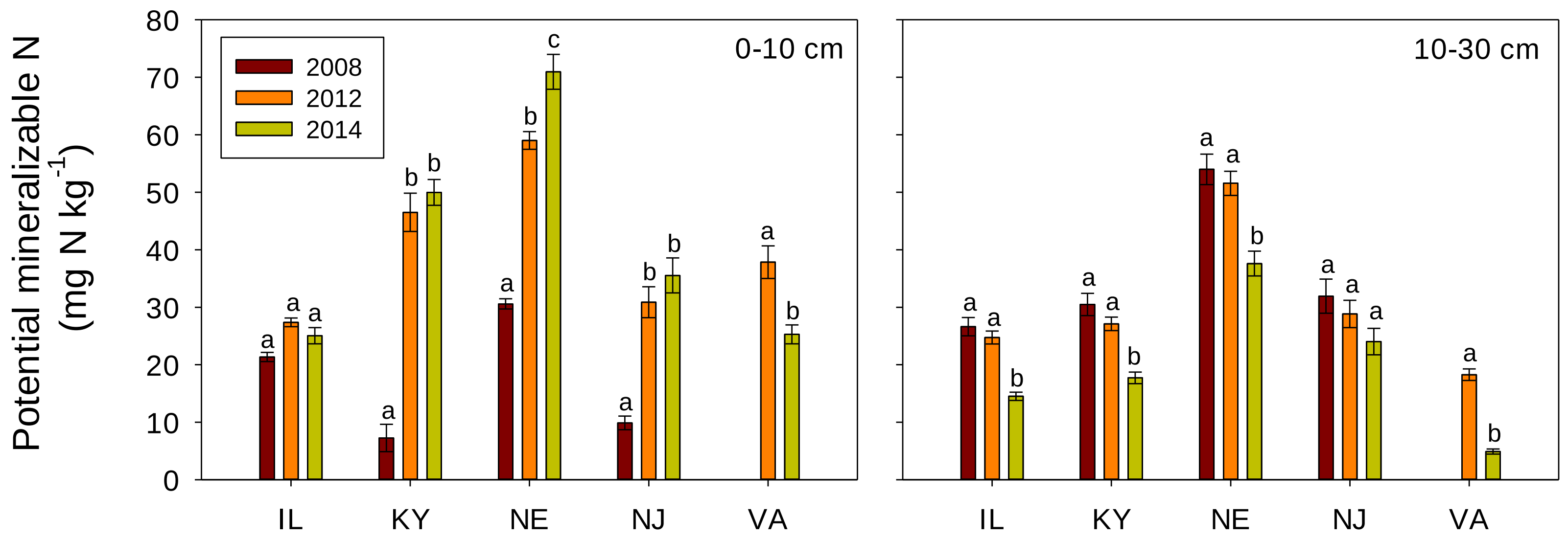


Figure 13. Average potential mineralizable N at soil 0-10 and 10-30 cm soil depths by sampling year. Error bars represent the standard error and letters significant difference between years.

Soil temperature, moisture and precipitation

Soil temperature at 10 cm varied from site to site as would be expected given the locations in the United States. For Kentucky, the annual soil temperature at 10 cm ranged from 12.2 ˚C during the 2014 growing season to 13.3 ˚C in the 2012 growing season with an average of 12.5 ˚C for all years. At the New Jersey site, growing season soil temperature ranged from 11.5 ˚C in 2014 to 12.4 ˚C in 2012 and from 13.8 ˚C in 2014 to 14.2 ˚C in 2012 at Virginia. Nebraska soil temperatures were the lowest of all states during winters of 2014 and 2015 but had 3 stable years with small variation with an average of 10.6 ˚C. The exception of this was Illinois that had lower soil temperatures from the summer of 2012 through the end of the 2013 winter than the rest of the sites. Illinois on average had a soil temperature of 9.7 ˚C at 10 cm of depth and it varied from 7.3 ˚C in 2012 growing year to 11.1 ˚C in 2014 growing year. During the growing season, Virginia had the highest soil temperatures and Nebraska and Illinois the lowest overall.

Soil moisture content varied at different depths with slightly higher volumetric water content at the deeper depths. At the 10 cm soil depth Illinois, Nebraska and Virginia reached a minimum soil moisture with 15.6, 19.8 and 19.8%, respectively. At the 30 cm depth, Illinois, Nebraska and New Jersey had minimum soil moisture contents of 16.6, 22.5 and 23.6%, respectively. At the deepest depth of 50 cm, Illinois, New Jersey and Virginia had the least soil moisture with 19.6, 28.8 and 24.0%, respectively.

The Kentucky site received more precipitation than any other site, exceeding its 30-year normal in 2013 and 2014 by 365 and 231 mm of precipitation respectively and consequently, due to its poor drainage soil texture it had the highest soil moisture. At Nebraska, that had been in the past the site with the best soil moisture retention (Davis et al., 2015), precipitation exceeded its 30-year normal average only by 67 and 129 mm respectively in 2013 and 2014 and as a consequence soil moisture was not as high as previous years and in the past three years the good drainage characteristic of their soil series might have played a major role.

Precipitation events have an influence in soil moisture, and we would expect that sites with greater precipitation would have higher soil moisture contents. New Jersey, Illinois and Virginia were expected to have the lowest soil moisture contents because of their sandy loam soil texture (Davis et al., 2015). Their sandy loam texture soils are characterized by good drainage properties compared to silt loam soils in Kentucky and Nebraska silty clay loam soils that because of the smaller clay particles, were expected to have poorer drainage than the rest of the sites.

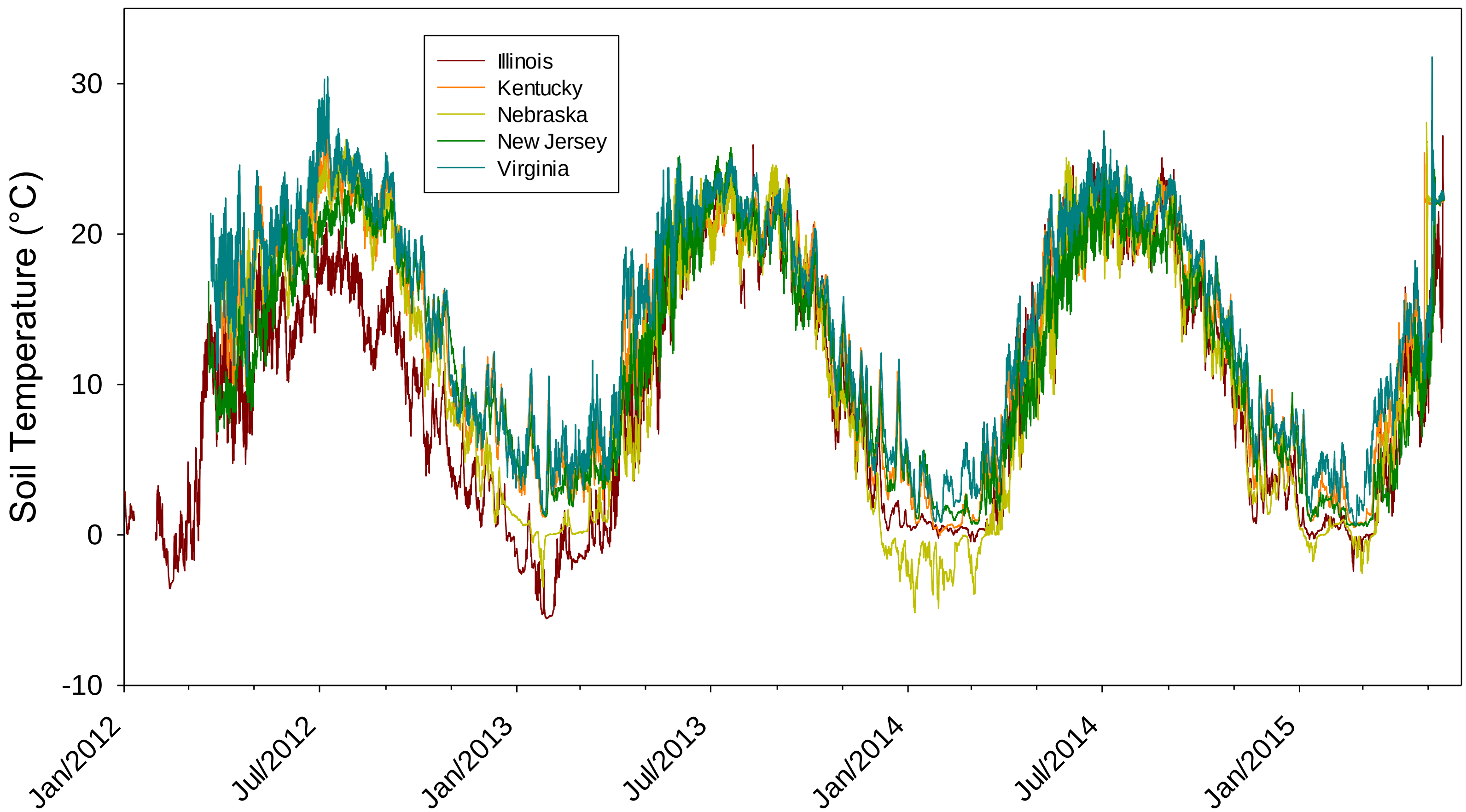


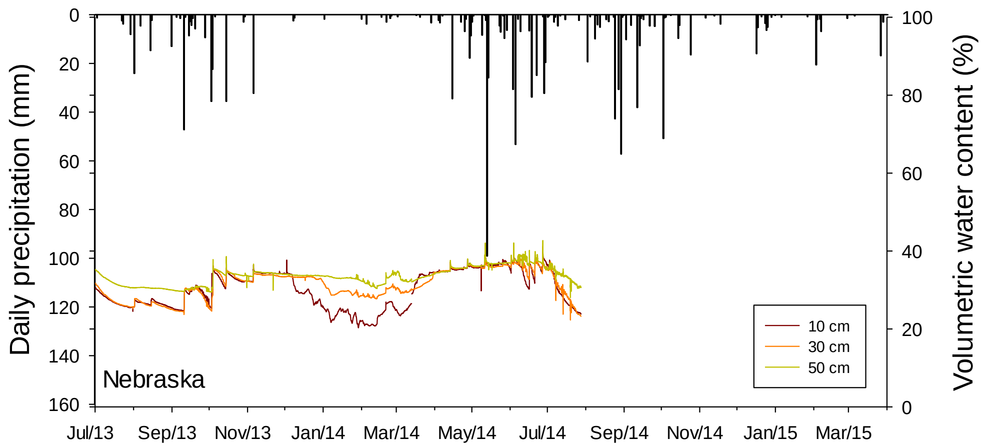
Figure 14. Soil temperature at 10 cm in all sites from 2012 to 2015.

Table 7. Total monthly precipitation (mm) at each site from 2008 to 2015.

	Precipitation (mm)																	
	IL									KY								
Month	2008	2009	2010	2011	2012	2013	2014	2015	30- Year normal	2008	2009	2010	2011	2012	2013	2014	2015	30- Year normal
January	54	6	24	22	71	61	24	28	52	112	110	77	52	90	113	59	47	81
February	114	44	26	61	44	67	65	20	51	146	65	41	158	79	39	120	76	81
March	79	77	71	40	46	20	35	36	70	165	61	29	119	112	136	74	189	103
April	58	172	67	179	69	169	96	78	96	152	122	59	323	59	124	152	290	91
May	139	118	75	108	48	100	169	142	116	112	153	253	164	92	144	138	53	134
June	181	85	212	87	51	102	161		106	91	132	117	81	41	192	142		113
July	176	139	98	21	0	128	152		110	75	192	154	125	204	231	82		118
August	19	108	86	38	174	7	43		92	55	115	15	92	55	131	243		83
September	186	42	42	66	77	14	81		83	36	150	16	152	138	42	111		74
October	73	188	25	60	101	102	87		80	39	147	32	112	33	158	114		80
November	29	97	95	124	28	29	56		89	64	24	113	195	45	62	60		90
December	107	78	48	68	55	48	43		64	153	102	64	113	167	142	84		100
Annual	1215	1154	868	874	763	847	1012	304	1009	1202	1373	968	1686	1112	1513	1379	655	1148

	NE									NJ								
	2008	2009	2010	2011	2012	2013	2014	2015	30- Year normal	2008	2009	2010	2011	2012	2013	2014	2015	30- Year normal
January	6	7	23	20	2	11	2	28	14	54	75	64	99	38	69	96	149	89
February	10	12	17	5	47	11	12	20	17	125	11	147	88	34	94	145	86	71
March	17	8	41	15	16	33	5	36	45	86	56	266	98	23	62	97	145	109
April	127	42	102	82	71	92	82	78	72	65	140	58	120	124	54	88	64	106
May	151	35	68	193	98	163	165	142	108	117	115	77	89	127	197	176	30	94
June	251	159	250	141	107	119	212		116	85	196	59	134	69	119	102		101
July	95	83	183	84	7	16	14		89	160	92	98	78	164	147	179		128
August	26	169	79	139	23	46	177		94	60	116	56	530	86	68	143		104
September	110	40	133	23	30	96	79		80	182	95	112	72	72	62	55		100
October	129	109	6	21	35	98	84		53	56	103	57	99	123	32	135		93
November	45	0	49	36	6	32	6		38	97	48	84	134	49	74	145		93
December	30	67	14	36	27	98	39		22	183	214	69	103	173	135	151		103
Annual	997	731	966	796	468	815	877	304	748	1269	1261	1147	1643	1082	1112	1512	474	1191

	VA								
	2008	2009	2010	2011	2012	2013	2014	2015	30- Year normal
January	23	103	189	60	60	232	155	53	96
February	59	35	82	27	26	53	55	44	76
March	65	105	134	106	147	73	76	77	103
April	161	84	44	106	90	95	141	92	90
May	134	166	133	125	85	120	71	47	103
June	57	120	61	50	102	187	40		95
July	105	107	87	57	114	164	115		114
August	123	184	124	63	50	117	212		97
September	89	56	137	173	125	38	94		113
October	64	67	89	87	70	54	90		84
November	87	234	70	144	11	103	79		91
December	115	163	64	76	99	155	80		87
Annual	1083	1424	1214	1074	978	1391	1208	313	1149



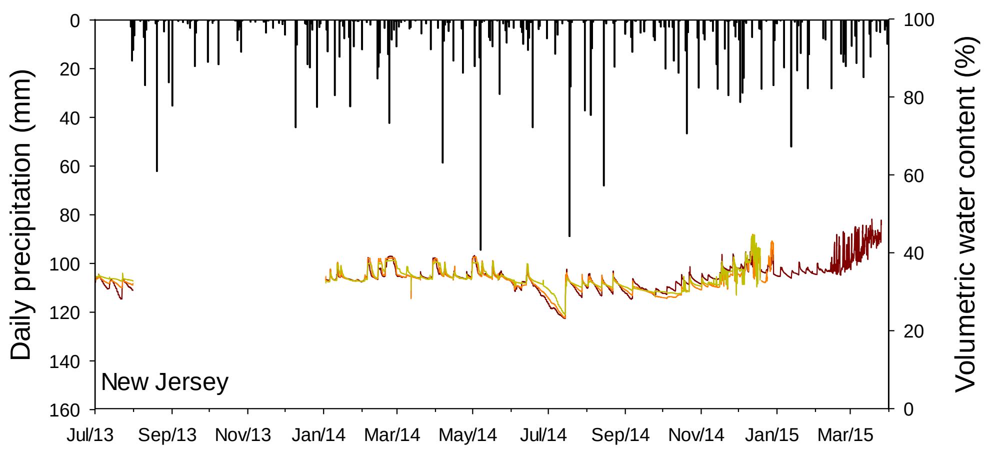


Figure 15. Volumetric soil water content at 10, 30, and 50 cm depths and daily precipitation at Nebraska and New Jersey from 2013 to 2015.

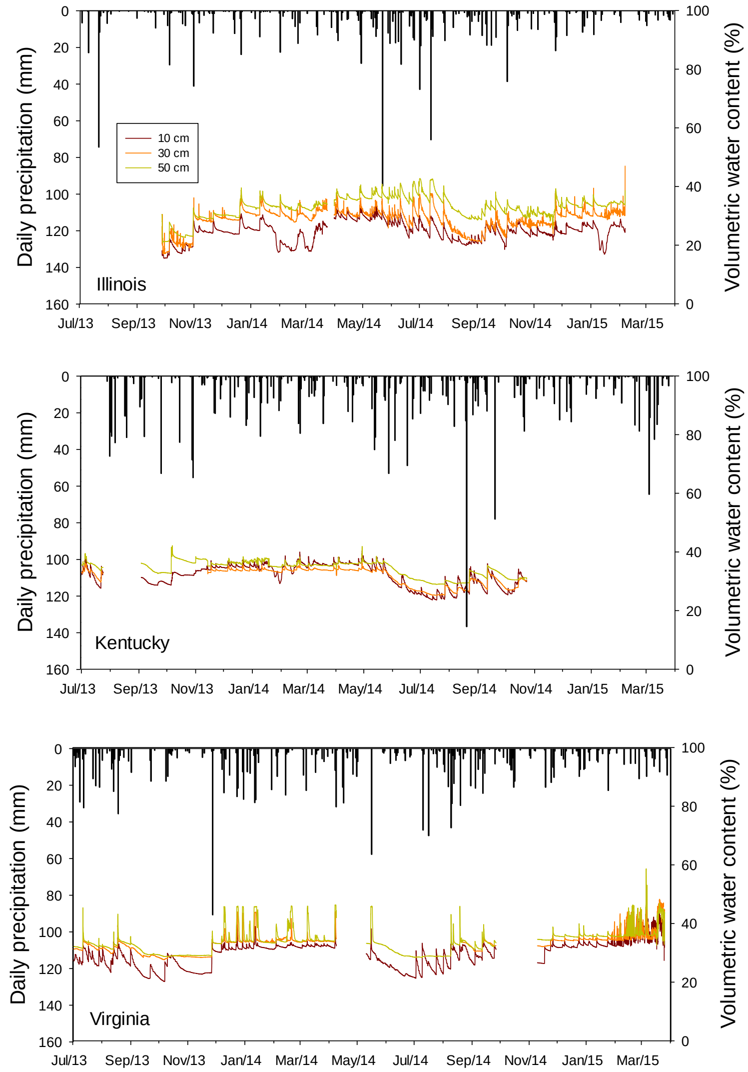


Figure 16. Volumetric soil water content at 10, 30, and 50 cm depths and daily precipitation at Illinois, Kentucky, and Virginia from 2013 to 2015.

Chapter 3: Conclusions

Carbon and N changes in SOM in soils under mature *M. x giganteus* are important to understand and evaluate the environmental impact of the biomass production of this crop. I monitored soil temperature and moisture and precipitation patterns at every site, biomass yields and loss of inorganic N through leaching. I also studied the role played by fertilizer rates in these losses, the changes in availability of C and the mineralization of N over the years as well as the possible relationship with microbial activity in soils.

During the 6th and 7th year after the establishment of *M. x giganteus*, a high yield potential has been shown in every site and fertilizer rate and most of the sites are still showing consistent yields. The greatest *M. x giganteus* yields on average were found at the Illinois and Nebraska sites, which had also the lowest precipitation and the lowest temperatures out of all sites which indicates that it does well in those environmental conditions. Overall, *M. x giganteus* shows response to N fertilization treatment even though 2 of the sites had a significant difference only when applying the higher fertilizer rate, and 1 was not affected at all by fertilizer application.

The results suggest that the fertilizer application might not be necessary during the establishment years and that fertilizers might help recover plants strength after severe weather conditions on less fertile soils. Nonetheless by the 7th year, 4 out of 5 sites were already showing improvement in yields with even the lowest level of fertilizer applied showing almost the same yields as the intensively fertilized if not better and this might suggest that as the crop matures fertilizer applications might become necessary.

M. x giganteus losses of inorganic N showed the same pattern in years 6 and 7 as during the previous years. Nitrate leaching increased with the fertilizer rate and even though in some sites the amount of nitrate leaching started to decrease, some of the sites had an increase in nitrate leaching during the 6^{th} and 7^{th} year but yields did not correspond to the amount of N supplied. The N provided to plants by fertilization might be getting lost instead through the water column instead and the rate of 60 kg N ha^{-1} might be sufficient since yields do not increase significantly with the 120 kg N ha^{-1} treatment. In addition to this, fertilization treatments did not have an effect on total C and N, potential mineralizable N, nor labile C. Ammonium leaching also showed a slight increase over the years as the crop got closer to a mature age. The ammonium leaching observed was present on sites that had lowest temperatures where higher yields were obtained.

Seven years after the change of soil use from turfgrass research to *M. x giganteus* there was no evidence that labile carbon has changed since the establishment year and that sites that had conventional crops had higher labile carbon than in the establishment year. Potential soil mineralization of N increased at 0-10 cm depth. Two of the sites that had the highest mineralization rate in 2014 transitioned from turfgrass research. These sites also had the highest microbial activity which could be explained that they have the highest labile C and the highest potential mineralizable N. To confirm that there is an increase in the rate of decomposition of SOM caused by microbial populations it would be recommended to compare results of this analysis over time.

Mature stands of *Miscanthus* x *giganteus* showed improvements in biomass production with the application of N fertilizer in this study but did not have an effect on labile carbon or mineralization of N. In addition, a negative environmental impact of fertilization was shown as N losses through leaching which increased with fertilization rate.

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